

Identification of quantitative trait loci (QTL) for plant structure, growth habit and yield in lentil

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Abstract

Lentil quantitative trait loci (QTL) related to plant structure (branches at first node, height of first node, total number of branches, plant height), growth habit (flowering time, pod dehiscence) and yield (number of seeds, seed weight, seed diameter) were located using a F_2 population of 113 individuals derived from the intersubspecific cross of *Lens culinaris* ssp. *culinaris* and *L. c.* ssp. *orientalis*. Several traits were found to be significantly correlated. Using interval and composite interval mapping a total of 23 QTL for nine quantitative traits were located. No QTL was identified for the number of F_3 seed produced. Six QTL were positioned respectively in linkage groups III and VI, and five QTL in linkage group I. Each remaining group included one or two QTL, except groups VII and IX where no QTL was found. The multiple QTL model explained more than 80% of the observed phenotypic variance with logarithm of the odds (LOD) scores above 10 for three of the quantitative traits analyzed (branches at first node, flowering time, and dehiscence). For the remaining traits the phenotypic variance explained was relatively low, between the 50% and 20%, and the LOD scores ranged between 4 and 8. The possible homology between some QTL and other previously described is discussed in relation to their chromosomal location.

Additional key words: genetic map, genetic markers, *Lens culinaris*, linkage map, mapping metric characteristics.

Resumen

Identificación de loci cuantitativos (QTL) para estructura de la planta, ciclo de crecimiento y rendimiento en lenteja

Se han cartografiado loci cuantitativos (QTL) de lenteja relacionados con la estructura de la planta (ramificaciones en el primer nudo, altura del primer nudo, número total de ramificaciones, altura de la planta), el ciclo de crecimiento (momento de floración, dehiscencia de vainas) y el rendimiento (número de semillas, peso de la semilla, diámetro de la semilla) utilizando una población F_2 derivada del cruzamiento intersubespecífico de *Lens culinaris* ssp. *culinaris* por *L. c.* ssp. *orientalis*. Se encontraron varias características significativamente correlacionadas. Aplicando los métodos de los marcadores flanqueantes simple y compuesto, se localizó un total de 23 QTL para nueve características cuantitativas. No se identificó ningún QTL para el número de semillas F_3 producidas. Se localizaron seis QTL respectivamente en los grupos de ligamiento III y VI, y cinco QTL en el grupo de ligamiento I. En cada uno de los restantes grupos de ligamiento se localizó uno o dos QTL, excepto en los grupos de ligamiento VII y IX, en los que no se hallaron QTL. El modelo múltiple de QTL explicó más de un 80% de la varianza fenotípica observada con valores del logaritmo decimal del cociente de verosimilitudes (LOD) por encima de 10 para tres de las características cuantitativas analizadas (ramificaciones en el primer nudo, momento de floración, dehiscencia de vainas). La varianza fenotípica explicada para el resto de las características fue relativamente baja, entre 50% y 20%, con valores LOD oscilando entre 4 y 8. Se discute la posible homología entre algunos QTL y otros previamente descritos en relación a su localización cromosómica.

Palabras clave adicionales: cartografiado de características métricas, *Lens culinaris*, mapa de ligamiento, mapa genético, marcadores genéticos.

Introduction

Lentil (*Lens culinaris* Medik.) is a self-pollinated diploid ($2n = 14$) legume with a relatively large genome

of 4,063 Mbp (Arumuganathan and Earle, 1991). This ancient pulse crop was most probably domesticated in the Fertile Crescent from *Lens culinaris* ssp. *orientalis* Boiss (Ladizinsky, 1993, 1999) at the dawn of agriculture. Lentil is a cool season grain legume normally grown in temperate semi-arid regions, usually in rotation with cereals, contributing to replenish soil nitrogen levels.

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Lentil seeds are valued as a food source of both high-quality plant proteins and fiber, in addition, the remaining plant residues can be used as animal feed and fodder.

Genetic linkage maps have recently become cornerstones in basic genetic analysis as well as in applied plant breeding. Biochemical and molecular markers have revealed that lentil has relatively low levels of genetic variation in comparison to other plant species (Alvarez *et al.*, 1997; Eujayl *et al.*, 1997; Ford *et al.*, 1997; Ferguson, 1998; Sonante and Pignone, 2001; Durán and Pérez de la Vega, 2004). The relatively reduced genetic variation and an insufficient amount of genetic information have until recently conditioned lentil genetic maps to include a relatively small number of markers, mainly isozymes and restriction fragment length polymorphisms (RFLPs) which covered an also relatively small portion of the lentil genome (Havey and Muehlbauer, 1989; Muehlbauer *et al.*, 1989; Tahir *et al.*, 1993). The use of more polymorphic markers based on the polymerase chain reaction (PCR), such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), and simple sequence repeat (SSR) has allowed for the construction of saturated or nearly saturated lentil genetic maps (Eujayl *et al.*, 1997, 1998; Rubeena *et al.*, 2003; Durán *et al.*, 2004; Kahraman *et al.*, 2004; Hamwieh *et al.*, 2005; Rubeena *et al.*, 2006). In order to maximize polymorphism for map construction in lentil, intersubspecific hybrid populations have sometimes been used (Havey and Muehlbauer, 1989; Eujayl *et al.*, 1997; Durán *et al.*, 2004).

So far, few studies have identified quantitative trait loci (QTL) in lentil (Muehlbauer *et al.*, 2006). Studies based on isozyme markers and single marker analysis detected three QTL, each one affecting respectively the variation in days to flower, days to maturity and plant height, whereas two or three loci were correspondingly identified for each of the traits biomass, seed yield, harvest index and seed weight (Tahir *et al.*, 1994; Tahir and Muehlbauer, 1995). Likewise, Kahraman *et al.* (2004), by means of interval mapping with a threshold of logarithm of the odds (LOD) 2.0, located some QTL related to winter hardiness in a genetic map based on RAPD, AFLP, and ISSR markers distributed in nine linkage groups (covering a total of 1,192 cM with an average distance between markers of 9.1 cM). They identified five independent QTL for winter survival which explained 33.4% of the total phenotypic variation,

and four QTL for winter injury accounting for 42.7% of the variation (Kahraman *et al.*, 2004). Recently, using genomic maps developed from two intraspecific F_2 populations, Rubeena *et al.* (2006) located a potentially common QTL and several common regions that contained markers significantly associated with resistance to ascochyta blight.

The objectives of this study were to locate lentil QTL related to plant structure (number of branches at the first node, height of the first node, total number of branches and plant height), growth habit (flowering time, pod dehiscence) and yield (number of seeds, average seed weight and average seed diameter), and also to obtain information on the effect (dominant, recessive, additive) of these quantitative traits.

Material and Methods

Plant material

An F_2 population of 113 individuals derived from the intersubspecific cross of *Lens culinaris* ssp. *culinaris* Medik. cv. 'Lupa' (a microsperma Spanish cultivar) as the female parent and *L. c.* ssp. *orientalis* Boiss (BG 16880) as the pollen donor (Durán *et al.*, 2004; Fratini *et al.*, 2004) was planted in the greenhouse at the beginning of February 1998, and grown under controlled conditions of $25 \pm 5^\circ\text{C}$ and natural daylight.

Quantitative and qualitative traits

The F_2 plants were scored individually for four different plant structure characteristics: number of branches at the first node, height of the first node (cm), total number of branches per plant and plant height (cm). Two different growth habit traits were considered: flowering time after an 80 day growth period (0 = flowering had not started; 1 = 1/2 plant was setting flower buds; 2 = 1/2 plant had buds in the 3/4 stage of petals to sepals with pollination taking place; 3 = 1/2 plant had open flowers with pod formation initiated) and pod dehiscence (0 = indehiscent; 1/4; 1/2; 3/4 dehiscent; 1 = completely dehiscent). Pod dehiscence was measured after plants had completely dried-out and were uprooted. In addition, three yield characteristics were evaluated: total number of F_3 seeds produced per F_2 plant, average F_3 seed weight (mg) and average F_3 seed diameter (mm). The average of F_3 seed weight

of each individual F_2 plant was established as the weight of the F_3 seed produced divided by the number of F_3 seeds. The average of F_3 seed diameter of each individual F_2 plant was determined by placing 30 F_3 seeds in a straight row without gaps, measuring the total length and dividing by 30.

In addition, the F_2 plants were also scored individually for four morphological traits: color (orange vs. yellow) of the cotyledon (*Yc*), presence or absence of anthocyanin in the stem (*Gs*), seed coat pattern or spotting (*Scp*) and ground color (brown vs. tan) of the seed (*Ggc*) (Vandenberg and Slinkard, 1990).

Molecular procedures, marker analysis and map construction

Methods regarding DNA extraction, PCR procedures, marker analyses and the construction of an inter-subspecific genetic map of *Lens* based on 158 molecular (71 RAPDs, 39 ISSRs, 83 AFLPs, and 2 SSRs) and 3 morphological markers segregating in the F_2 population were described by Durán *et al.* (2004). The genetic maps consisted of 10 linkage groups covering 2,172.4 cM with an average distance between markers of 15.87 cM (Durán *et al.*, 2004). Linkage groups in this work are indicated with Roman numerals and linkage groups referred to other publications are indicated by Arabic numerals.

Statistical and QTL analysis

A correlation study between all the different quantitative traits was undertaken using the software package STATISTICA 98 for Windows (StatSoft, Tulsa, OK, USA). QTL analysis was carried out following the method of interval mapping (Lander and Botstein, 1989), performed by both MAPMAKER/QTL (Paterson *et al.*, 1988; Lincoln *et al.*, 1992) and Windows QTL Cartographer (Wang *et al.*, 2005), in addition the method of composite interval mapping (Zeng, 1993, 1994) performed only under Windows QTL Cartographer was also applied. A LOD score of 3.0 was chosen as the threshold for considering a putative QTL peak. Peaks had to appear simultaneously at corresponding sites along the linkage map using both methods implemented by both software programs. QTL positions were established by the peak LOD score produced by Windows QTL Cartographer composite

interval mapping. Multiple peaks within 30 cM were considered as a single QTL (Kearsey and Pooni, 1996). Up to 3 putative QTL were fixed using the «sequence» and «scan» commands of MAPMAKER/QTL to detect new and eliminate spurious QTL. The «try» feature of the «sequence» command of MAPMAKER/QTL was used to find the best fitting genetic model of the individual QTL. The sequence of all the fixed QTL specifying their genetic model and the «map» command of MAPMAKER/QTL were applied to determine the multiple QTL model LOD score, percentage of the phenotypic variance (σ^2) explained, and mean (μ) of the trait.

Results

Parental phenotypes

The two parents, cultivar 'Lupa' and the wild *L. c. orientalis*, differed widely for the set of quantitative traits analyzed. In general, the wild sub-species *orientalis* is a short and extremely branched plant with a prostrate growth habit, and whose first node is located at ground level; it flowers soon after an 80-day growth period and is completely dehiscent, producing small seeds of a reduced weight. On the other hand, 'Lupa' is tall, has an erect structure with occasional branching and the first node is positioned well above ground level (traits especially important for mechanical harvest); it flowers after approximately 110 days and is completely indehiscent, producing larger and heavier microspermatophyte seeds.

Correlation analysis of quantitative traits

A correlation study between all the different quantitative traits analyzed was carried out (Table 1). Several significant to very highly significant correlations were found, although in no case did the correlation coefficient exceed 0.41. The number of branches at the first node was positively correlated to the height of the first node (0.369). These two morphological traits were not significantly correlated with any other trait analyzed. The total number of F_3 seeds produced by the F_2 plants was found to be positively correlated to flowering time (0.405), to the total number of branches produced (0.409) and to the seed size (0.338). Likewise, seed weight was positively correlated to the total number

Table 1. Significant correlations between traits in *Lens culinaris* × *L. orientalis* F₂ population. For trait abbreviation see Fig. 1

Trait	Number branches 1 st node							
Height 1 st node (cm)	0.369 ***	HN ^b						
Total number of branches	— ^a	—	TB					
Plant height (cm)	—	—	0.204*	PH				
Flowering time (0, 1, 2, 3)	—	—	—	−0.223*	FT			
Dehiscence (0, 1/4, 1/2, 3/4, 1)	—	—	—	0.260 **	−0.191*	DH		
Number of seeds	—	—	0.409 ***	—	0.405 ***	—	NS ^b	
Seed weight (mg)	—	—	0.251 **	—	—	—	0.260 **	SW
Seed diameter (mm)	—	—	—	—	0.280 **	—	0.338 ***	0.340 ***

^a Not significant. ^b NS: number of seeds. * P < 0.05; ** P < 0.01; *** P < 0.001.

of branches produced (0.251). Flowering time was negatively correlated to plant height (−0.223) and to the level of dehiscence (−0.191), while positively to the number of seeds produced (0.405) and to seed size (0.280), but there was no significant correlation to seed weight. Finally, seed weight and seed size were positively correlated but the correlation was lower than expected (0.340).

Multiple QTL models

A total of 23 QTL were located in the intersubspecific genetic map of *Lens* for the nine quantitative traits studied (Figure 1 and Table 2). No QTL with a significant LOD threshold above 3.0 were detected for the total number of F₃ seeds produced by the individual F₂ plants, this quantitative trait had the lowest degree of homoscedacity and normality. Two to four QTL were identified and located for the remaining eight metric traits. Figure 1 shows that a total of five QTL for five different quantitative traits were located in the first linkage group (I), six QTL for six different traits were located in the third linkage group (III) and six QTL for five characteristics were found in the fifth linkage group (V). The remaining linkage groups either had one or two QTL, with exception of linkage groups seven (VII) and nine (IX) which lacked associations with quantitative traits.

In this study, the multiple QTL model yielded better results, higher LOD scores and explains a higher proportion of the phenotypic variance, for traits (number of branches at the first node, flowering time and dehiscence) which can be classified into a lower number of phenotypic classes (four to five) and which had a

higher degree of homoscedacity and normality compared to traits with a more continuous distribution (Table 2).

With regard to the genetic effects of the quantitative traits studied, it was found that while the QTL related to the height of the first node had an additive effect, two out of the three related to plant height were recessive (always referring to the alleles from *orientalis*). On the other hand, of those related to the number of branches at the first node and the total number of branches per plant, the two located in linkage group III were recessive while the remaining were dominant. Both flowering time and dehiscence were associated to dominant and recessive alleles. For seed size the genes of an additive effect seemed to be predominant while seed weight was related to additive and recessive genes (Table 2). The QTL with the highest effect on flowering time, located at 147 cM in linkage group IV, had an allelic effect that was the opposite of what was predicted from the parental phenotypes.

In the case of three quantitative traits analyzed, namely, the number of branches at the first node, flowering time and dehiscence, the multiple QTL model explained more than 80% of the phenotypic variance observed with LOD scores well above 10 (Table 2). All these traits were classified in a relatively low number of phenotypic classes (four or five). For the remaining traits the phenotypic variance explained was relatively low, between 50% and 20%, and LOD scores ranged between 4 and 8.

Discussion

A total of 23 QTL were identified and located in the intersubspecific genetic map of *Lens* (Fig. 1 and Table 2).

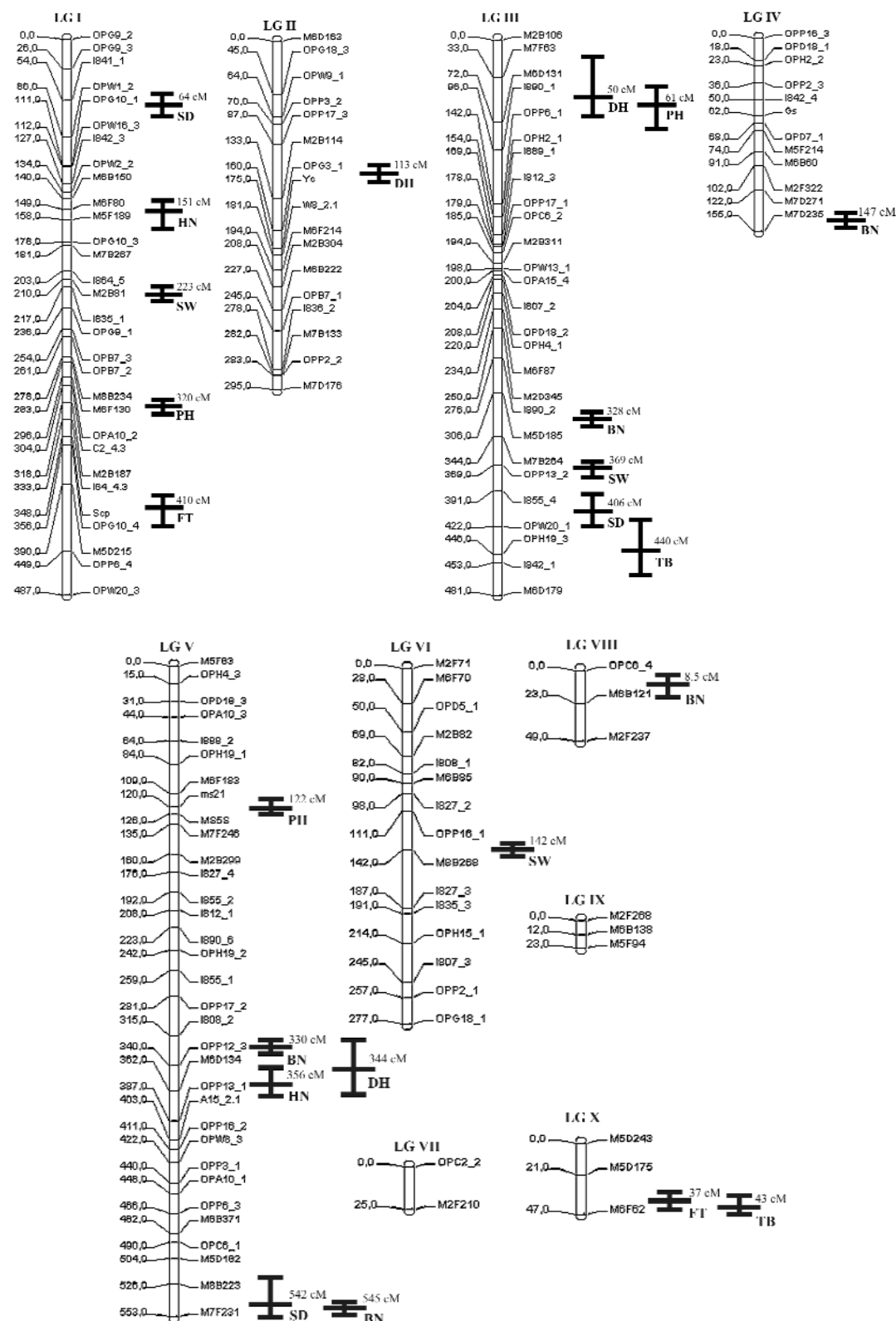


Figure 1. Intersubspecific genetic map of *Lens* detailing QTL position with confidence interval of minus one LOD. BN: number of branches at the 1st node. HN: height of the 1st node. TB: total number of branches. PH: plant height. FT: flowering time. DH: dehiscence. SW: seed weight. SD: seed diameter.

Table 2. QTL location^a and genetic effect^b according to the multiple QTL model

Linkage group	Number branches 1 st node	Height 1 st node (cm)	Total number of branches	Plant height (cm)	Flowering time (0, 1, 2, 3)	Dehiscence (0, 1/4, 1/2, 3/4, 1)	Seed weight (mg)	Seed diameter (mm)
I	—	151 (141-169) cM additive w = -1.0588 d = 0	—	320 (314-328) cM recessive w = -2.9407 d = 2.9407	410 (400-426) cM dominant w = 0.2027 d = 0.2027	—	223 (217-227) cM recessive w = 1.0726 d = -1.0726	64 (50-78) cM dominant w = 0.0787 d = 0.0787
II	—	—	—	—	—	113 (105-119) cM dominant w = 0.0211 d = 0.0211	—	—
III	328 (322-334) cM recessive w = 0.0962 d = -0.0962	—	440 (416-462) cM recessive w = 4.3139 d = -4.3139	61 (41-77) cM additive w = 6.6220 d = 0	—	50 (20-68) cM recessive w = -0.2735 d = 0.2735	369 (365-377) cM additive w = -1.8505 d = 0	406 (392-422) cM additive w = -0.1111 d = 0
IV	—	—	—	—	147 (143-149) cM recessive w = -0.9549 d = 0.9549	—	—	—
V	330 (322-336) cM dominant w = -0.0502 d = -0.0502 545 (543-551) cM dominant w = -0.0769 d = -0.0769	356 (344-370) cM additive w = -0.7056 d = 0	—	122 (114-126) cM recessive w = -3.2200 d = 3.2200	—	344 (318-364) cM dominant w = 0.1024 d = 0.1024	—	542 (520-553) cM additive w = -0.1463 d = 0
VI	—	—	—	—	—	—	142 (136-148) cM recessive w = -1.5722 d = 1.5722	—
VIII	8.5 (2.5-16.5) cM dominant w = -0.9186 d = -0.9186	—	—	—	—	—	—	—

Table 2 (cont). QTL location^a and genetic effect^b according to the multiple QTL model

Linkage group	Number branches 1 st node	Height 1 st node (cm)	Total number of branches	Plant height (cm)	Flowering time (0, 1, 2, 3)	Dehiscence (0, 1/4, 1/2, 3/4, 1)	Seed weight (mg)	Seed diameter (mm)
X	—	—	43 (35-47) cM dominant w = 1.7561 d = 1.7561	—	37 (33-43) cM dominant w = 0.0375 d = 0.0375	—	—	—
Total	4	2	2	3	3	3	3	3
QTL	LOD 27.37 $\sigma^2 = 91.7\%$ $\mu = 3.066$	LOD 7.63 $\sigma^2 = 33.3\%$ $\mu = 3.265$	LOD 4.07 $\sigma^2 = 54.0\%$ $\mu = 8.788$	LOD 6.84 $\sigma^2 = 38.2\%$ $\mu = 37.873$	LOD 20.91 $\sigma^2 = 90.4\%$ $\mu = 2.591$	LOD 11.12 $\sigma^2 = 81.3\%$ $\mu = 0.648$	LOD 4.16 $\sigma^2 = 18.2\%$ $\mu = 27.804$	LOD 5.63 $\sigma^2 = 37\%$ $\mu = 3.978$

^a Map position (minus one LOD value confidence interval). ^b d and w indicate the respective values of the QTL weight and dominance, always referred to the allele from *L. orientalis*. σ^2 and μ indicate the explained variance and the mean of the trait according to the multiple QTL model.

A high number of QTL were located in this study in linkage groups I, III and V. This uneven QTL distribution agrees with previous results by Tahir *et al.* (1994). They described that linkage groups 1, 2, 5 and 7 of their lentil gene map (Tahir *et al.*, 1993) seemed to contain a greater number of quantitative traits than other genomic regions represented by linkage groups 3 and 4. Two to four QTL were related to eight out of the nine quantitative traits analyzed in this study except for the number of F₃ seeds produced per F₂ plant for which no QTL was detected. This could possibly be due to the fact that the number of seeds is a complex trait of a composite nature influenced by several factors such as the number of seeds per pod, flowers per peduncle, peduncles per branch, branches per plant, and the length of the vegetative plant phase among others. As can be observed in Table 1, significant correlations were found between the total number of F₃ seeds produced and both the total number of branches per plant (0.409) and flowering time (0.405). A delayed flowering extends the vegetative growth phase and increases the number of branches per plant which in turn increases the photosynthetic surface and enhances seed production. On the other hand, the relatively low correlation between seed weight and seed size (diameter) could be due to other components of the weight not measured in this work, such as seed filling and/or seed storage components.

Since no consensus genetic map of lentil exists, it is not always easy to compare the results obtained to different works in relation to the chromosomal location of the QTL analyzed. Nevertheless, some QTL could

be tentatively identified between different studies. Using the method of single marker analysis and isozymes, a QTL related to days to flowering was previously located in linkage group 1, while QTL related correspondingly to days to maturity and to plant height were both located in linkage group 5 (Tahir *et al.*, 1993; Tahir and Muehlbauer, 1995). Linkage groups 1 and 5 by Tahir and co-workers must respectively correspond to our current linkage groups IV and I (Durán *et al.*, 2004), since they share in that order the location of the *Gs* (green stem) and *Scp* (seed coat pattern) loci. In this study, a QTL for flowering time and a QTL for plant height were also located in the corresponding linkage groups IV and I, respectively. Sarker *et al.* (1999) reported that the trait days to flowering was controlled by a single recessive gene (*Sn*) as well as by a polygenic system. The *Sn* gene was found to be linked to the morphological marker *Scp*. Thus, it seems probable that the *Sn* gene and our QTL for flowering time located in linkage group I (classified as dominant, and at 62 cM from *Scp*) were the same. Furthermore, an additional dominant QTL related to flowering time was found in this study and located in linkage group X (Fig. 1 and Table 2).

A QTL related to plant height was located by Tahir and Muehlbauer (1995) in linkage group 5. Likewise, we have located a QTL for plant height in the corresponding linkage group I. A recent study (Kumar *et al.*, 2005) established linkage relationships between the traits leaf color (*Gl*), whole plant pubescence (*Pub*), number of leaflets (*Hl*) and a major gene for plant height (*Ph*). Kumar *et al.* (2005) state that the whole

plant pubescence (*Pub*) locus corresponds to the pubescent pod (*Pep*) locus described by Sarker *et al.* (1999) and found to be linked to the seed coat pattern (*Scp*) locus in linkage group 5. Therefore, the QTL previously related to plant height by Tahir and Muehlbauer (1995) in linkage group 5 or the QTL found in the present study in the corresponding linkage group I could possibly coincide with the plant height locus (*Ph*) described by Kumar *et al.* (2005). Furthermore, two additional QTL related to plant height were located in this study in linkage groups III and V, respectively (Fig. 1 and Table 2).

Tahir and Muehlbauer (1995) also located three QTL for seed weight in linkage groups 1, 4 and 5, respectively. Likewise, a total of three QTL related to seed weight were also identified in the present study and located in linkage groups I, III and VI (Fig. 1 and Table 2). Therefore, at least the QTL located here in linkage group I coincides with one of the QTL in linkage group 5 described by Tahir and Muehlbauer (1995). Further correspondences can not be determined since the lack of common markers does not allow for the identification of further homologous linkage groups. Likewise, the correspondence between QTL located in the same linkage group is only tentative since Tahir and Muehlbauer (1995) could not precisely locate the position of these quantitative loci within the lentil linkage groups.

Even though in certain lentil crosses indehiscence has been reported as monogenic (Muehlbauer *et al.*, 1989; Tahir and Muehlbauer, 1994) and a qualitative gene (*Pi*) has been located in linkage group 4 of the lentil genome (Tahir *et al.*, 1993), in this study it was analyzed as a quantitative trait since dehiscence-indehiscence did not segregate according to a 3:1 ratio and intermediate phenotypes were identified. A total of three QTL for dehiscence were respectively located in linkage groups II, III and V. According to the location of the cotyledon color gene (*Yc*), linkage group II of this study corresponds to linkage group 2 of the genetic map by Tahir *et al.* (1993). Dehiscence or pod shattering has been described to be controlled in *Glycine max* by either three QTL (Saxe *et al.*, 1996) or one major gene and a few minor QTL (Bailey *et al.*, 1997).

One of the QTL related to flowering time had an allelic effect that was the opposite to that predicted from the parental phenotypes. In other segregating plant populations obtained from crosses between the cultivated form and its wild relative, up to 46% of the QTL had allelic effects opposite to those predicted from the parental phenotypes (Frery *et al.*, 2003).

To sum up, this is the first study in lentil to map quantitative traits related to plant structure, growth habit and yield, by means of interval mapping (Lander and Botstein, 1989) and composite interval mapping (Zeng, 1993, 1994) in a dense genetic map which includes several kinds of genetic markers (RAPDs, AFLPs, ISSRs, and SSRs). QTL for several previously described traits have been mapped in this study (plant height, flowering time, seed weight). Moreover, QTL for a new set of traits have been described and located (number of branches at first node, height of first node, total number of branches, dehiscence, seed diameter). A total of 23 QTL have been located which influence the expression of the 9 quantitative traits examined. However, the conclusions are specific to the F₂ population examined under the greenhouse environmental conditions in which the measurements were taken. Further research using RIL obtained from this and additional segregating populations, and additional markers, such as microsatellites and single nucleotide polymorphisms, will be needed to obtain a more precise QTL location in the lentil genetic map.

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